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Constant Phycobilisome Size in Chromatically Adapted Cells of the Cyanobacterium *Tolypothrix tenuis*, and Variation in *Nostoc* sp.[1]

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ABSTRACT

Phycobilisomes of *Tolypothrix tenuis*, a cyanobacterium capable of complete chromatic adaptation, were studied from cells grown in red and green light, and in darkness. The phycobilisome size remained constant irrespective of the light quality. The hemidiscoidal phycobilisomes had an average diameter of about 52 nanometers and height of about 33 nanometers, by negative staining. The thickness was equivalent to a phycocyanin molecule (about 10 nanometers). The molar ratio of allophycocyanin, relative to other phycobiliproteins always remained at about 1:3. Phycobilisomes from red light grown cells and cells grown heterotrophically in darkness were indistinguishable in their pigment composition, polypeptide pattern, and size. Eight polypeptides were resolved in the phycobilin region (17.5 to 23.5 kilodaltons) by isoelectric focusing followed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Half of these were invariable, while others were variable in green and red light. It is inferred that phycoerythrin synthesis in green light resulted in a one for one substitution of phycocyanin, thus retaining a constant phycobilisome size. *Tolypothrix* appears to be one of the best examples of phycobiliprotein regulation with wavelength. By contrast, in *Nostoc* sp., the decrease in phycoerythrin in red light cells was accompanied by a decrease in phycobilisome size but not a regulated substitution.

Complementary chromatic adaptation is a response where the quality of light regulates the synthesis of certain phycobiliproteins in some cyanobacteria. Generally, green light promotes PE[4] synthesis and suppresses PC synthesis (3, 5, 7, 18, 23). Depending on the species, chromatic adaptation can occur to various degrees, that is, PE may be completely, or only partially absent. Along with the PE reduction, the size of the PBS may be reduced or it may remain unchanged (4, 5, 10, 21, 22, 24).

We have long been interested in the photoregulation of PE and PC in *Tolypothrix tenuis* (18, 19) and thus undertook a study of the PBS morphology with changes in light growth conditions. In *Tolypothrix*, as in many cyanobacteria and a number of red algae the PBS are hemidiscoidally shaped (fan-shaped) (8). A hemiscoidally shaped phycobilisome, as first proposed for the red alga *Rhodella* (15) and with a significantly similar structure in cyanobacteria (6) consists of a core, and about 4 to 6 peripheral arms. It has been shown that all of the APC is in the core. By EM, the core has three central discs in face view, but from side views it was determined that the core actually consists of six hexamers (1). The peripheral arms radiating from the core contain the PC and, when present, the PE. The pigment composition, expressed as molar ratios is usually in good agreement with the morphological manifestations, but there are some notable exceptions. For example, in *Anacystis* which has been well documented by Glazer *et al.* (11), the core has only two pairs of hexamers. Furthermore, according to Siegelman and Kycia (22), the APC hexamers vary between 6 and 9 depending on the species. In constructing models of hemidiscoidally shaped PBS, it is generally assumed that the PBS thickness is equivalent to the diameter of at least one hexamer (6, 10, 11, 24). In actuality, the thickness of PBS is rarely determined because it cannot be done from negatively stained preparations. Embedded-thylakoid sections are required.

Prompted by our interest in the interrelationship of the photosystems and their related light harvesting antennae, the PBS, we first needed to know if the PBS number can be estimated from the phycobiliprotein content. For this it was necessary to ascertain whether or not the PBS size was variable or constant with changing light conditions. From each light condition we determined the phycobiliprotein content. We also determined the PBS shape and size (diameter, height, and thickness) in sectioned cells, and determined the diameter and height of PBS by negative staining. We similarly examined *Nostoc* sp., which does not undergo complete chromatic adaptation, and where the pigment changes have been described (2, 9, 13, 24). Our results show that with changing wavelength the pigment composition changes in both species, but the PBS size remains constant in *Tolypothrix* while altering in *Nostoc*.

MATERIALS AND METHODS

Cultures of *Tolypothrix tenuis* (M29 Algal Collection of the Institute of Applied Microbiology, University of Tokyo) were grown at 30°C in medium enriched with glucose, casamino acids, and vitamins which supports heterotrophic growth (14). The cultures, in fermentation flasks with continuous slow shaking, were either in complete darkness, or in green light or red light at about 1×10^{15} photons/cm^2. The green light source consisted of fluorescent lamps (GE, Cool White) and a No. 2092 Rohm and Haas Co. green plexiglass filter. The red light source consisted of incandescent lamps covered with red filter, No. P-14 Gelatin Products (Glen Cove, NY). Cells in late exponential growth were used for EM and for the isolation of PBS as per Gantt *et al.* (9) and modified for *Tolypothrix* by increasing the Triton X-100 to

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[4] Abbreviations: PE, phycoerythrin; APC, allophycocyanin; PC, phycocyanin; PBS, phycobilisomes; IEF, isoelectrofocusing.

3%, and the initial ionic condition to 0.9 M phosphate buffer as in Ohki and Gantt (19).

Nostoc sp. cultures were grown at 37°C in continuous red or green light at the levels as above, as well as with a 3-fold increase of the above. (Phycobilisome composition and size did not vary significantly over this intensity range.) The red light source for *Nostoc* consisted of a P-14 Gelatin Products filter over Sylvania lamps (F48T12/CWX/VH0) which had an enhanced output in the red spectrum. The green light source consisted of a P-38 Gelatin Products filter over Sylvania lamps (F48T12/D/VH0). Since the cultures grew very rapidly they were diluted every 24 to 48 h to avoid self shading. For both algae the green light conditions had maximal output at about 540 nm, and the red light condition had maximal output at about 620 to 660 nm.

For EM spheroplasts were prepared, as detailed in Ohki and Gantt (19). This was necessary to obtain defined PBS in sections. Fixation, in 1.5% glutaraldehyde was followed by postfixation in 1% osmium tetraoxide. Negative staining with 1% uranyl sulfate on isolated PBS was carried out after fixation on the electron microscope grid in 0.7% glutaraldehyde. Phycobilisome size was determined from electron micrographs with a 25 to 100 sample size from each treatment. Fewer samples (18–20) were available for determination of PBS in linear arrangement along the thylakoid (Fig. 3).

Two-dimensional gel electrophoresis on *Tolypothrix* PBS was performed utilizing urea-IEF in the first dimension and SDS-PAGE in the second dimension. For the first dimension a 9.2 M urea-gel, with Servalyt^R T 4-9 ampholytes, was prepared according to O'Farrell (17) (without Nonidet) and cast as a slab (0.75 mm thick on a sheet of Servagel-Fix^R) for use in an LKB Multiphor apparatus. The pH gradient was established in 1 h at 10 V/cm^2 15°C. Samples were applied in presoaked filter paper pads at about pH 4.5, and electrofocusing at the conditions above was continued for 1.5 to 2 h. Slices of the gel, about 1 cm wide, were cut after electrofocusing, some were stained with Coomassie blue according to Rhigetti and Drysdale (20), and others were stored frozen at −80°C. For electrophoresis in the second dimension, Laemmli's (16) SDS-PAGE system was used with an acrylamide gradient of 9 to 15%, and 1.5 mm thickness. The stacking gel was cast with a special slot former which allowed inclusion of an IEF gel slice next to a PBS sample. The gel slice, after coming to room temperature, was equilibrated in soaking solution for 30 min and then embedded along its vertical edge with agarose as suggested by O'Farrell (17). Electrophoresis was performed for 18 h at 12 amp at 20°C. Gels were fixed in 12% TCA for 20 min and stained in 0.25% Coomassie blue R-250, 30% methanol, and 10% acetic acid.

Separation of PBS polypeptides on SDS-PAGE was also carried out on a 9 to 15% acrylamide gradient according to Laemmli (16).

RESULTS AND DISCUSSION

Chromatic adaptation in *Tolypothrix tenuis* occurs with the result that PE is always absent in cells grown in darkness and in red light, and is only present in green (or white) light (18). Its response to light is thus similar to cyanobacteria of group III as defined by Tandeau de Marsac (23) and with the phycobiliprotein changes as shown by Bryant and Cohen-Bazire (4, 5). *Nostoc* sp. under our conditions appears to be a type II, with a reduction but not a complete absence of PE.

In *Tolypothrix*, light is not required for synthesis of fully functional PBS. Phycobilisomes from red light and dark grown cells are indistinguishable from each other by their polypeptide composition (Figs. 1 and 2, Table I) and their morphology (19). With green light induction of PE the composition changes. The polypeptide pattern of PBS from cells under varied light conditions is illustrated in Figure 1. The blue phycocyanobilin containing polypeptides were present in the mol wt range of 17.5 to 22 kD (Table I) which is typical for PC and APC. At least seven additional polypeptides (uncolored on SDS-PAGE) were present in these PBS. The largest, with an apparent mol wt at about 93 kD is the presumed terminal pigment and possible anchor protein between the PBS and the thylakoid. Those at 39, 38, and 31 kD are attributable to PC and APC stacking. In green light the uncolored polypeptides increased by at least two, at about 37 and 35 kD, which appeared simultaneous with PE and are thus attributable to PE stacking. Two phycoerythrobilin bands were also present at 23.5 and 22 kD.

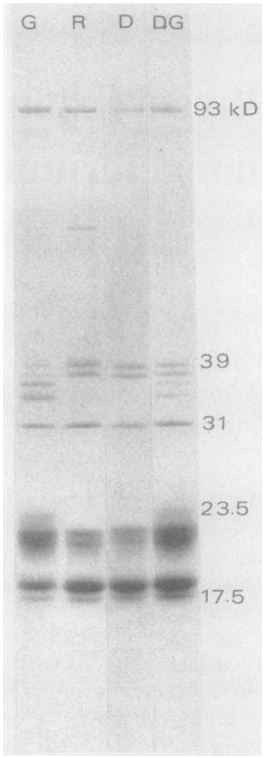

FIG. 1. Polypeptides on SDS-PAGE of *Tolypothrix tenuis* phycobilisomes from cells grown in various wavelength conditions and darkness: (G), green light; (R), red light; (D), dark. Dark plus a 15 min green light pulse every 24 h (DG). Each lane was loaded with about 100 μg protein. Chromophore containing polypeptides: 93 kD and 23.5 to 17.5 kD.

Eight polypeptides were resolved in the 17.5 to 23.5 kD region by IEF (9.2 M urea) followed by SDS-PAGE (Fig. 2). Three blue colored areas were evident in all PBS, with presence of a red area in PE containing PBS. In the constant blue region, four polypeptides (PC and APC) were present on staining in constant alignment at about pI 5.2 to 5.6 (18–17.5 kD). These are probably part of the invariable portion of the PBS. Four other polyeptides were variable, as seen by a comparison of red light and green light PBS (upper right and lower right, Fig. 2, respectively). Examination of the gels prior to staining clearly showed that each pink polypeptide had one pI form at about 5.6 to 6.0 in green light PBS. Under the same conditions one blue polypeptide was present at about pI 5, and a second at about pI 6.3. In red light, and dark, the additional ones were at about 5 and 6.5 (21–21.5 kD, and 18 kD respectively). In PBS of dark grown cells exposed to short green light pulses, the PE polypeptides were of intermediate density (lower left). The linker polypeptides 31 to 39 kD did not resolve into additional pI forms, and since the 93 kD polypeptide did not migrate into the gel, its pI was not determined. Analysis of the phycobiliprotein extracts from *Fremyella* (Calothrix 7601) grown under red light, and green light by Bryant (4) resolved into six polypeptides; all were blue in red

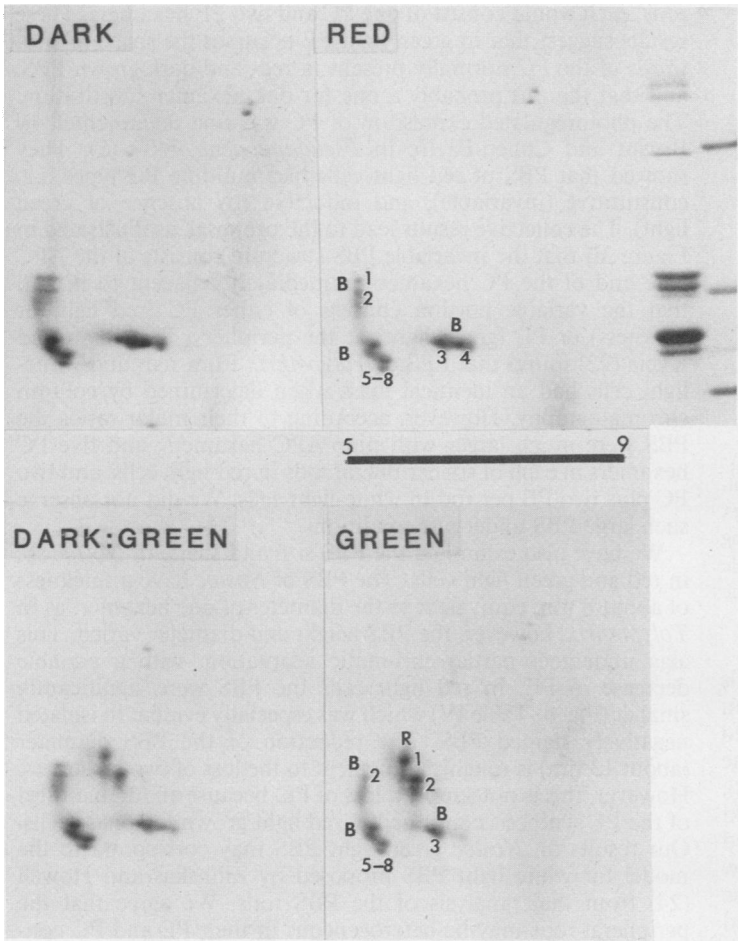

FIG. 2. Polypeptides of *Tolypothrix* phycobilisomes separated by two-dimensional electrophoresis. First separation was by IEF in 9.2 M urea, then on SDS-PAGE. The polypeptide color indicated by letters was present prior to Coomassie blue staining: B, blue colored polypeptides; R, red colored polypeptides, others were colorless. Shown attached to the red light grown phycobilisome pattern (upper right) are the corresponding polypeptide bands on SDS-PAGE and mol wt markers (66,000 kD, BSA on top; 14,200 kD, lysozyme on bottom). The black line indicates the IEF gradient pI 5 to 9.

Table I. *Polypeptides of Tolypothrix Phycobilisomes Isolated from Cells Grown under Various Wavelengths and in Darkness*

Green Light Grown		Red Light Grown		Dark Grown		Dark Grown Plus Green Pulse	
Apparent mol wt	Stained protein[a]	Apparent mol wt	Stained protein[a]	Apparent mol wt	Stained protein[a]	Apparent mol wt	Stained protein[a]
kD	%	*kD*	%	*kD*	%	*kD*	%
93	3.0	93	3.0	93	2.5	93	3.0
(70–62)[b]		(70–62)					
61	4.5	61	3.5	(70–57)	3.5	(65–57)	4.0
(59–57)		(59–57)					
39.0	1.5	39.0	4.0	39.0	4.0	39.0	2.5
38.0	1.5	38.0	3.0	38.0	3.0	38.0	2.0
37.0	2.5					37.0	1.5
35.0	2.5					35.0	2.0
31.0	3.0	31.0	4.0	31.0	3.5	31.0	4.0
23.5		22.0		22.0		23.5	
22.0		21.5		21.5		22.0	
21.5	80.5	21.0	80.0	21.0	81.0	21.5	80.0
18.0		18.0		18.0		18.0	
17.5		17.5		17.5		17.5	
14.5	0.5	14.5	1.0	14.5	1.0	14.5	0.5
13.5	0.5	13.5	1.5	13.5	1.5	13.5	0.5

[a] Per cent of total Coomassie Blue stained protein bands determined by scanning SDS-PAGE gels. [b] Values in parentheses indicate faint bands.

light cells, while two were pink in green light cells. His preliminary examination of *Tolypothrix* (Calothrix 7101) suggested that the results were similar to *Fremyella*. By our IEF analysis two additional polypeptides have been resolved. Since they are in the blue region (prior to SDS) they may contain chromophores, otherwise they may be uncolored linkers. Awareness of the additional pair of invariable polypeptides is important in gene product analysis.

Phycobilisome size and shape in *Tolypothrix* were determined from electron micrographs as in the coordinates shown in Figure 3A and from views such as previously shown in *Tolypothrix* (19) and illustrated for *Nostoc* in Figure 4. In *Tolypothrix*, the PBS

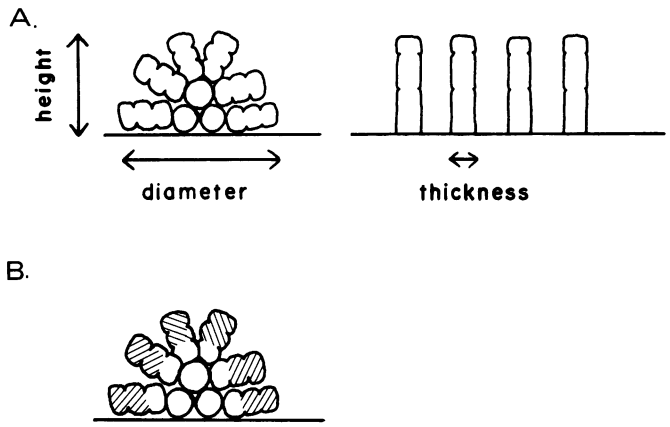

FIG. 3. A, Diagramatic representation of phycobilisomes on the thylakoids in face view on left, and linearly arranged side-views on right. Phycobilisome dimensions were determined from sections of *Tolypothrix* (also as illustrated in Fig. 4 of *Nostoc*). B, In this face view representing a *Tolypothrix* phycobilisome, the shaded region suggests the variable portion (PE, or inducable PC), and the unshaded region suggests the invariable portion.

were constant whether PE was present or absent, or whether cells had been grown in light or darkness. This is evident from measurements of PBS by EM in sectioned cells and of isolated and negatively stained PBS (Table II). By negative staining the PBS had the typical central core (3 units when seen in face-view), surrounded by 5 to 6 peripheral stacked rods [see Fig. 3 in Ohki and Gantt (19)]. The peripheral stacked rods usually contained three double discs. In green light, the PBS size in broad face view was 54 ± 4 nm (diameter) by 34 ± 2 nm (height). These dimensions are virtually the same as those from red light and dark grown cells (19). In sectioned cells the PBS appeared uniform in size (Table II). In sectioned cells, the PBS dimensions are typically less due to dehydration required in resin-embedding. Only from sectioned cells can one determine the thickness of hemidiscoidally shaped PBS and their longitudinal array, and thus ascertain their overall size. The thickness and spacing along the thylakoid membrane also appeared to be relatively uniform, *i.e.* under all light conditions tested they retained the same size and shape. In fact, the thickness closely corresponds to the diameter of one hexameric disc (PE or PC).

The pigment content, using the extinction coefficients for APC, PC, and PE, as determined by Hattori and Fujita (12) for *Tolypothrix*, yielded the molar ratios in Table III. From these ratios, the known PBS morphology, and assuming APC to be constant it can be predicted that PBS of red- and dark-grown cells have six APC hexamers in the core, 18 PC in 6 peripheral rods (about three hexamers per rod), consistent with electron microscopic images (19). In green-grown cells, the PBS would have the same APC core, and assuming 6 homogenous peripheral rods, each would consist of one PC and two PE hexamers. These results suggest that in green light PE occupies the space of two-thirds of the PC normally present in red- and dark-grown PBS, and that there is probably a one for one hexamer substitution. The photoregulated expression of PC was first documented by Bryant and Cohen-Bazire in *Pseudanabaena* 7409 (5). They showed that PBS of red light cells had multiple PC types, *i.e.* constitutive (invariable), and inducible (by absence of green light). The collective results lead to the proposal, as illustrated in Figure 3B that the invariable PBS structure consists of the APC core and of the PC hexamers immediately adjacent to it, and that the variable portion consists of either PC (red light or darkness) or PE (green light) at the periphery. Siegelman and Kycia (22) found that PBS of *Tolypothrix* from red- and white-light cells had an identical mass when determined by column chromatography. However, according to their molar ratios the PBS were much larger with nine APC hexamers, and five PC hexamers in each of six peripheral rods in red-light cells, and two PC plus two PE per rod in white-light cells. We did not observe such large PBS under our conditions.

We have also examined the PBS size and shape in *Nostoc* sp. in red and green light cells. The PBS of *Nostoc* have a thickness of about 8 nm, equivalent to the diameter of one hexamer, as in *Tolypothrix*, however, the PBS height and diameter varied. This alga undergoes partial chromatic adaptation, with a variable decrease in PE. In red light cells the PBS were significantly smaller (Fig. 4, Table IV) which was especially evident in isolated negatively stained PBS. The reduction of the PBS diameter (about 13 nm) is roughly equivalent to the loss of two hexamers. However, this is not simply a loss of PE, because about one-third of the PE synthesis continued in red light-grown log phase cells. Our results on *Nostoc* green light PBS may correspond to the model for white light PBS proposed by Zilinskas and Howell (24) from their analysis of the PBS rods. We agree that the peripheral rods may be heterogeneous in their PE and PC content, but equally likely is the possibility that many PBS are devoid of PE altogether. PE variability seems to be caused by a number of factors. Whereas numerous laboratories have been using *Nostoc* sp. from one original clone, PE decrease has been found as a result of lowered temperature (2, 9), and spontaneous mutation (13) as well as light quality (24). By comparison, *Tolypothrix* is relatively stable with a predictable wavelength response.

CONCLUSION

In *Tolypothrix*, light is not required for PBS production. *Tolypothrix* cells undergoing chromatic adaptation produce PE only in green light. Red light was not required for induction of PC in *Tolypothrix*. Green light induced PE synthesis, and inhibited two-thirds of PC normally synthesized in red light or darkness. Phycobilisome size remained constant in cells grown under varied wavelengths, and in darkness. Two-thirds of the PC content was replaced by PE (in green light).

Table II. *Tolypothrix tenuis Phycobilisome Dimensions*

Growth Condition	Isolated Negatively Stained		In Cells Embedded and Sectioned			
	Diameter	Height	Diameter	Height	Thickness	PBS/1000 nm (longitudinal)[a]
light condition	*nm*			*nm*		
Green light cells	54 ± 4[b]	34 ± 3	40 ± 4	27 ± 6	10 ± 2	52
Red light cells	52 ± 2	34 ± 2	43 ± 3	28 ± 2	11 ± 2	63
Dark grown cells	51 ± 2	33 ± 2	42 ± 3	24 ± 4	9 ± 2	58

[a] Number of phycobilisomes in side views along 1000 nm longitudinal thylakoid section. [b] Mean ± SD.

Table III. *Tolypothrix Phycobiliprotein Content of Phycobilisomes, and Predicted Hexamer Content*

	Molar Ratio			Hexamers/Phycobilisome		
	PE	PC	APC	PE	PC	APC
Red light	0	315 ± 40	100	0	19	6
Dark	0	298 ± 30	100	0	18	6
Green light	206 ± 16	140 ± 20	100	12	8	6

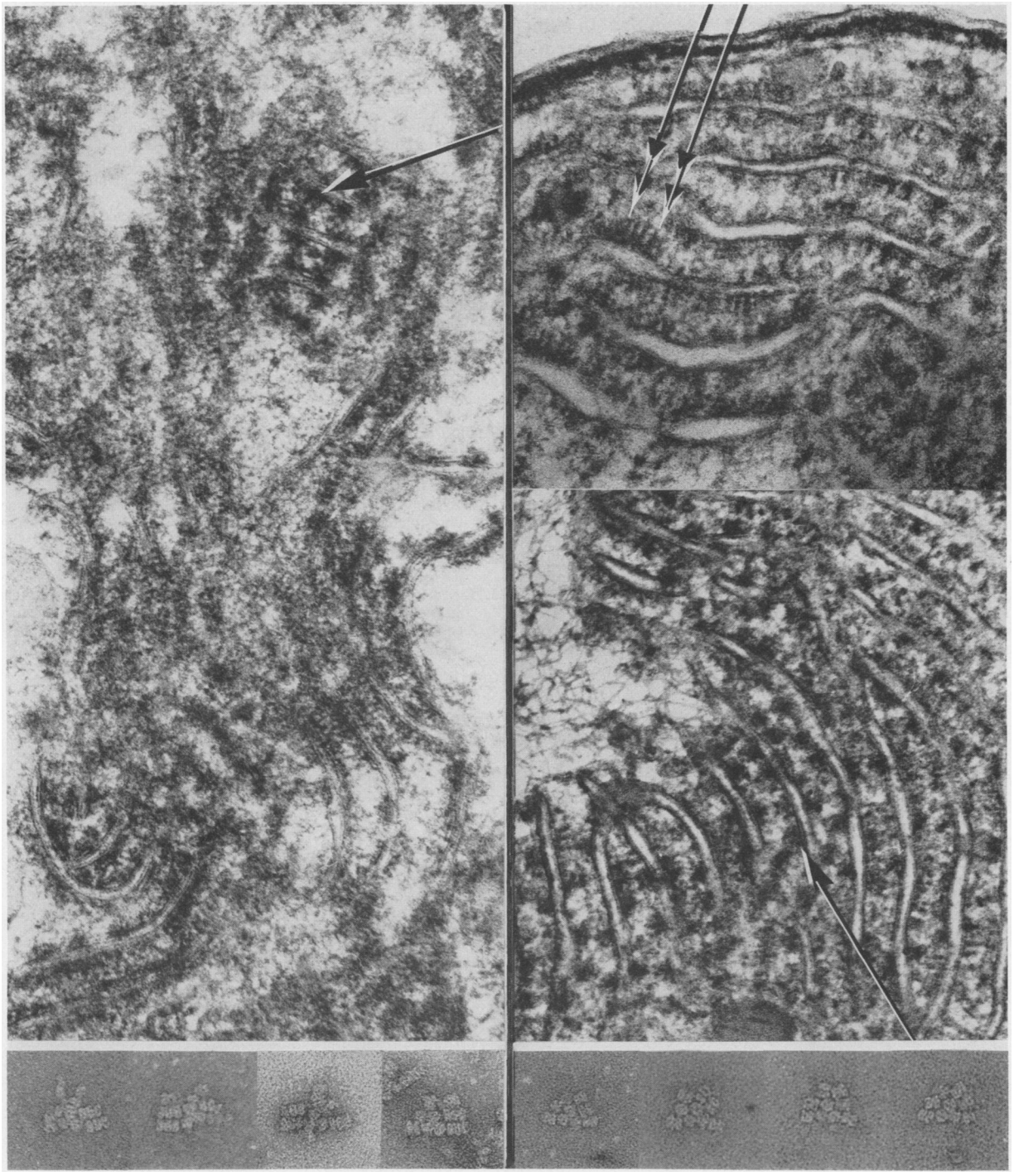

FIG. 4. Phycobilisomes of *Nostoc* sp. On the left side are shown sections and isolated negatively stained phycobilisomes from green-light grown cells. On the right side are sections and phycobilisomes from red-light grown cells. Phycobilisomes in broad face view are designated by single arrows, and in side view by double arrows. (Sections enlarged × 129,000, negative stained views × 220,000).

Table IV. *Nostoc sp. Phycobilisome Dimensions*

Growth Condition	Isolated Negatively Stained		In Cells Embedded and Sectioned		
	Diameter	Height	Diameter	Height	Thickness
light condition	*nm*			*nm*	
Green light cells	55 ± 3[a]	34 ± 4	38 ± 3	28 ± 3	8 ± 2
Red light cells	42 ± 2	30 ± 3	33 ± 3	27 ± 2	8 ± 2

[a] Mean ± SD.

Nostoc PBS in red light were smaller than in green light, and the cells appear to lack the capacity for PC substitution. In both species, the thickness of PBS is equivalent to the diameter of about one hexamer (certainly less than two). For meaningful estimates of the PBS number, from phycobiliprotein content, the total size (diameter, height, thickness) must be known and can only be accurately determined if the PBS size is constant, or when the variation in size is controllable, which is possible in *Tolypothrix* but not in *Nostoc*.

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